Act1 intron (McElroy *et al.*, 1990). As shown below, the maize *hsp70* intron is useful in the present invention.

As noted above, the 3′ non-translated region of the chimeric plant genes of the present invention contains a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3′ end of the RNA. Examples of preferred 3′ regions are (1) the 3′ transcribed, non-translated regions containing the polyadenylate signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene and (2) plant genes such as the pea ssRUBISCO E9 gene (Fischhoff *et al.*, 1987).

5.15.2 PLANT TRANSFORMATION AND EXPRESSION

A chimeric transgene containing a structural coding sequence of the present invention can be inserted into the genome of a plant by any suitable method such as those detailed herein. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, *e.g.*, by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and Eur. Pat. Appl. Publ. No. EP0120516. In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery *via* microprojectile bombardment, and transformation using viruses or pollen (Fromm *et al.*, 1986; Armstrong *et al* , 1990; Fromm *et al.*, 1990).

5.15.3 CONSTRUCTION OF PLANT EXPRESSION VECTORS FOR *CRY** TRANSGENES

For efficient expression of the *cry** variants disclosed herein in transgenic plants, the gene encoding the variants must have a suitable sequence composition (Diehn *et al.*, 1996).

To place a *cry** gene in a vector suitable for expression in monocotyledonous plants (*i.e.* under control of the enhanced Cauliflower Mosaic Virus 35S promoter and link to the *hsp70* intron followed by a nopaline synthase polyadenylation site as in U. S.

Patent No. 5,424,412, specifically incorporated herein by reference), the vector is digested with appropriate enzymes such as *Nco*I and *Eco*RI. The larger vector band of approximately 4.6 kb is then electrophoresed, purified, and ligated with T4 DNA ligase to the appropriate restriction fragment containing the plantized *cry** gene. The ligation mix is then transformed into *E. coli*, carbenicillin resistant colonies recovered and plasmid DNA recovered by DNA miniprep procedures. The DNA may then be subjected to restriction endonuclease analysis with enzymes such as *Nco*I and *Eco*RI (together), *Not*I, and *Pst*I to identify clones containing the *cry** gene coding sequence fused to the *hsp70* intron under control of the enhanced CaMV35S promoter).

To place the gene in a vector suitable for recovery of stably transformed and insect resistant plants, the restriction fragment from pMON33708 containing the lysine oxidase coding sequence fused to the *hsp70* intron under control of the enhanced CaMV35S promoter may be isolated by gel electrophoresis and purification. This fragment can then be ligated with a vector such as pMON30460 treated with *Not*I and calf intestinal alkaline phosphatase (pMON30460 contains the neomycin phosphotransferase coding sequence under control of the CaMV35S promoter). Kanamycin resistant colonies may then be obtained by transformation of this ligation mix into *E. coli* and colonies containing the resulting plasmid can be identified by restriction endonuclease digestion of plasmid miniprep DNAs. Restriction enzymes such as *Not*I, *Eco*RV, *Hin*dIII, *Nco*I, *Eco*RI, and *Bgl*II can be used to identify the appropriate clones containing the restriction fragment properly inserted in the corresponding site of pMON30460, in the orientation such that both genes are in tandem (*i.e.* the 3′ end of the *cry** gene expression cassette is linked to the 5′ end of the *nptII* expression cassette). Expression of the Cry* protein by the resulting vector is then confirmed in plant protoplasts by electroporation of the vector into protoplasts followed by protein blot and ELISA analysis. This vector can be introduced into the genomic DNA of plant embryos such as maize by particle gun bombardment followed by paromomycin selection to obtain corn plants expressing the *cry** gene essentially as described in U. S. Patent No. 5,424,412, specifically incorporated herein by reference. In this example, the vector was introduced *via* cobombardment with a hygromycin resistance conferring plasmid into

immature embryo scutella (IES) of maize, followed by hygromycin selection, and regeneration. Transgenic plant lines expressing the Cry* protein are then identified by ELISA analysis. Progeny seed from these events are then subsequently tested for protection from susceptible insect feeding.

6.0 REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U. S. Patent 4,237,224, issued Dec. 2, 1980.

U. S. Patent 4,332,898, issued Jun. 1, 1982.

U. S. Patent 4,342,832, issued Aug. 3, 1982.

U. S. Patent 4,356,270, issued Oct. 26, 1982.

U. S. Patent 4,362,817, issued Dec. 7, 1982.

U. S. Patent 4,371,625, issued Feb. 1, 1983.

U. S. Patent 4,448,885, issued May 15, 1984.

U. S. Patent 4,467,036, issued Aug. 21, 1984.

U. S. Patent 4,554,101, issued Nov. 19, 1985.

U. S. Patent 4,683,195, issued Jul. 28, 1987.

U. S. Patent 4,683,202, issued Jul. 28, 1987.

U. S. Patent 4,757,011, issued Jul. 12, 1988.

U. S. Patent 4,766,203, issued Aug. 23, 1988.

U. S. Patent 4,769,061, issued Sep. 6, 1988.

U. S. Patent 4,797,279, issued Jan. 10, 1989.

U. S. Patent 4,800,159, issued Jan. 24, 1989.

U. S. Patent 4,883,750, issued Nov. 28, 1989.

U. S. Patent 4,910,016, issued Mar. 20, 1990.

U. S. Patent 4,940,835, issued Feb. 23, 1990.

U. S. Patent 4,965,188, issued Oct. 23, 1990.